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HYDROGEN-RATED SYSTEM FOR *IN VITRO* STUDIES AT PRESSURE: EXPERIMENTAL VALIDATION

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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A special hyperbaric perfusion apparatus was constructed to study the neurophysiologic effect of high pressure and the pharmacology of various gaseous agents using the isolated nerve terminal (synaptosome) tissue preparation. This study was performed to validate the performance of this apparatus in comparison with an alternate device constructed of different materials. This was motivated by theoretical considerations and some anecdotal indications that stainless steel, utilized in the fabrication of the hyperbaric device, might possibly be toxic to synaptosomes. Time course of K^+-stimulated release of [^{3}H] GABA from guinea pig cerebrocortical synaptosomes was the same for the stainless steel hyperbaric superfusion device as for an apparatus made of plastic. As a practical matter, stainless steel construction material does not appear to affect synaptosome function.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, DDH, Pub. No. (NIH) 85-23.

INTRODUCTION

Synaptosomes are isolated nerve terminal endings that maintain many of the functional properties of the intact presynaptic neuron. As such, the synaptosome preparation is a useful model for study of presynaptic physiology. Synaptosomes have been used to investigate the effects of high pressure on nerve terminal Ca^{2+} uptake (1) and on K^{+}-depolarized (2-8) or A23187 Ca^{2+} ionophore stimulated (9) neurotransmitter release.

One motivation for such studies would be the delineation of how pressure affects the central nervous system (CNS) to cause the high pressure nervous syndrome (HPNS). This is a debilitating condition that can impair the performance and threaten the safety of human divers at depths greater than 600 feet sea water (fsw). It is manifested by tremor, arousal deficits, and EEG changes (10,11). In animals exposed to even greater pressures, HPNS progresses to convulsions and death.

Many narcotic/anesthetic drugs ameliorate HPNS (12). Included among these are a number of gaseous agents. The pharmacologic activity of some of these gases becomes apparent only under hyperbaric conditions. Nitrogen, the predominant constituent of air, is a noteworthy example. The addition of 5-10% N_2 to a breathing mixture has been used as a countermeasure to HPNS in attaining record depths in human diving (13). Thus, the pharmacology of breathing gasses is most relevant to the problem of high pressure effects on the CNS.

There has been a resurgence of interest in the utility of hydrogen as a component of breathing mix for deep diving (14). Low density and other considerations confer practical advantages to the use of H_2. Moreover, hyperbaric H_2 produces narcosis, ameliorates HPNS, and is intermediate in potency between He and N_2 (15,16). Human experiments confirm the utility of H_2 in alleviating HPNS (17,18), but potentially adverse occurrences including apneic episodes have been observed (19). Consequently, the CNS action of hyperbaric H_2 becomes an object of intense scrutiny.

In order to investigate the pharmacology of various hyperbaric gases including H_2, Imbert and colleagues (20) designed a special apparatus to saturate liquids with defined gas mixtures at high pressure. These solutions are used to perfuse *in vitro* cellular or organelle preparations, which are thereby exposed to dissolved gas at the partial pressure of each component in the gas mixture.

The apparatus was constructed to include use with the synaptosome preparation. Two separate gas-saturated physiologic electrolyte solutions, containing low K^+ and high K^+ respectively, are employed to perfuse the synaptosomes. Switching perfusion from a low K^+ to a high K^+ solution induces neurotransmitter release, presumably by depolarizing a transmembrane potential. Using this technique, the time course of neuro-transmitter release can be studied, subject to experimental manipulations of pressure and gas mixture.

One issue of concern with respect to the new apparatus was whether the materials from which it was built could inadvertently affect neurotransmitter release from the synaptosomes. Specifically, there was concern about stainless steel used in the construction of the device. Although many experiments have been performed using stainless hardware (*viz.* 21,22), there is anecdotal information that stainless steel might possibly have a deleterious effect on secretion, based on experience with a glioma cell line in tissue culture (23).

Theoretical considerations suggest additional reasons for concern regarding stainless steel. Ions of Fe and other transition metals can participate in the formation of biologically damaging free radicals from species that arise during the respiratory reduction of oxygen to water (24,25). For example, escape of O_2^- from the cytochrome complex and its dismutation to H_2O_2 sets the stage for the so-called Haber-Weiss reaction (26,27). In the first step of Haber-Weiss, Fe^{3+} is reduced to Fe^{2+} by O_2^-. In the second step, the Fenton reaction, H_2O_2 oxidizes Fe^{2+} back to Fe^{3+} with production of $HO^{\cdot}$. As the most potent oxidizing agent known, $HO^{\cdot}$ reacts rapidly and indiscriminantly, and can denature a variety of biological molecules. The free radical mediated peroxidation of unsaturated fatty acid, for example, initiates a chain reaction of lipid peroxidation that is the basis of rancidification of fat. Such Fe-dependent peroxidation has been shown to impair GABA uptake and release by synaptosomes (28).

Leeching of trace amounts of metal ions such as Ni^{2+} or Cd^{2}, present either accidentally or by design in steel alloy, poses another theoretical concern. In micromolar concentrations, these ions block the membrane voltage-gated Ca^{2+} channels that mediate secretion, neurotransmitter release, and other cellular processes (29-31). This consideration is all the more urgent in view of electrophysiologic evidence that pressure affects conductance at voltage-gated Ca^{2+} channels (32-34). The voltage-gated Ca^{2+} channel is a candidate locus of the salient actions of pressure on the nervous system. Thus, it would be most desirable not to confound observations on the neurophysiological effects of high pressure with covert interactions arising from the pharmacological effects of inorganic metal ions.

To evaluate the possibility that stainless steel or other unidentified toxic materials in the superfusion apparatus might affect synaptosome function, a second device was constructed using plastic. Experiments were performed in both devices at normobaric pressure. For each device, synaptosomes were exposed to perfusion media for either 5 min or 25 min prior to stimulation. Results for the separate devices were then compared. The rationale was that active products derived from the material used to build the intended hyperbaric apparatus would become evident by differential results following prolonged exposure. The experiment also addressed the issue of whether results might be affected by the duration of prestimulation perfusion, as for example during a 25-minute interval required for chamber compression.

MATERIALS AND METHODS

Experiments with synaptosomes were performed using either the hyperbaric superfusion apparatus as described (20) or an alternate apparatus. Fluid reservoirs for the hyperbaric superfusion apparatus were constructed in part using 316-grade stainless steel, while those for the alternate apparatus were all plastic. For either apparatus, the perfusion media were saturated by bubbling with a 21% O_2, balance He, gas mixture. The reservoirs were pressurized to 10 psig to provide the driving force to perfuse the tissue preparation. Perfusion media were maintained at 37°C.

To prepare synaptosomes, two young Hartley guinea pigs (*Cavia porcellus*) weighing ~600 g were quickly decapitated and the brains rapidly removed. The cerebral cortical mantle was separated by blunt dissection from the underlying structures and washed in ice-cold 0.32M (isotonic) sucrose (ICIS) buffered with 5 mM tris(hydroxymethyl)aminomethane hydrochloride (TRIS), pH 7.4. Then the tissue was minced, placed in 20 ml ICIS, and dispersed in a 55-ml Potter-Elvehjem homogenizer over ice using six up-and-down strokes over a motor-driven rotating Teflon pestle. The crude homogenate was placed in Sorvall 30-ml polycarbonate Oak Ridge tubes and spun at 3000 RPM, $\omega^2 dt$ 1.44 x 10^7 rad^2/s, on a SS34 rotor in a Sorvall RC28S centrifuge refrigerated at 4°C. The supernatant was then similarly centrifuged at 13,000 RPM, $\omega^2 dt$ 6.26 x 10^8 rad^2/s. The pellet was resuspended in ICIS and further homogenized using a hand-held 10-ml Potter-Elvehjem device. It was then equally divided among six Sorvall 17 ml polyallomer centrifuge

tubes by layering onto a discontinuous Ficoll density gradient system prepared as 7 ml 7.5% Ficoll (w/v in ICSS, pH 7.4 with methylamine) atop 6 ml 13% Ficoll (same), all kept ice-cold. The tubes were placed in an AH-627 swinging bucket rotor and centrifuged at 26,000 RPM, $\omega^2 dt$ 1.16 x 10^{10} rad^2/s, in a Sorvall OTD75B centrifuge refrigerated to 4°C. The material at the density interface of the Ficoll gradient was recovered, resuspended in 40 ml ICIS to wash out Ficoll, and centrifuged in the SS34/RC28S at 11,000 RPM for 10 min. The resulting pellet was resuspended in 40 ml ice-cold perfusion buffer (ICPB, in mM, NaCl 145, KCl 5, $MgCl_2$ 3.7, KH_2PO_4 1.2, TRIS HCl 20, Glucose 10, aminooxyacetic acid 0.1; pH 7.4) to wash out glucose and centrifuged as before. The final pellet was resuspended in 20 ml ice cold storage buffer (same as ICPB but with addition of 10 μM GABA). This final tissue suspension was stored in an ice-melt water bath. Figure 1 and Figure 2 are electron micrographs of the material of this suspension following glutaraldehyde fixation. The figures demonstrate ultrastructural features that are typical of nerve terminals such as intraterminal vesicles positioned near synaptic densities, and they are comparable to previously published (35) electron micrographs of synaptosomes.

Six experiments were performed each day for four days using the alternate superfusion apparatus. This was then repeated using the hyperbaric superfusion apparatus. All experiments on any given day were performed within 6 h of completing the synaptosome preparation.

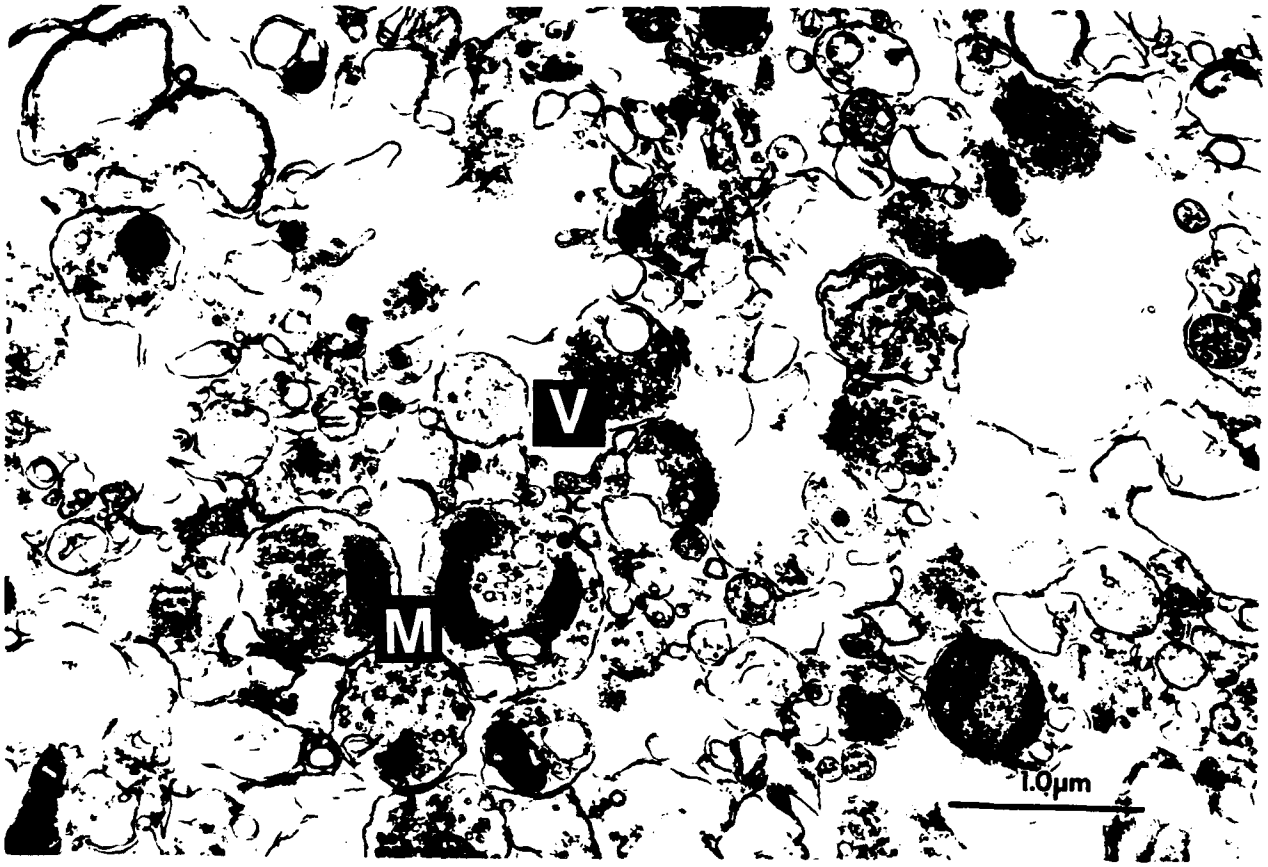

Figure 1. Transmission electron micrograph of tissue preparation, X 26,600. The preparation is seen to contain discrete membrane-bound fragments that harbor occasional mitochondria (M) and numerous small vessicles (V).

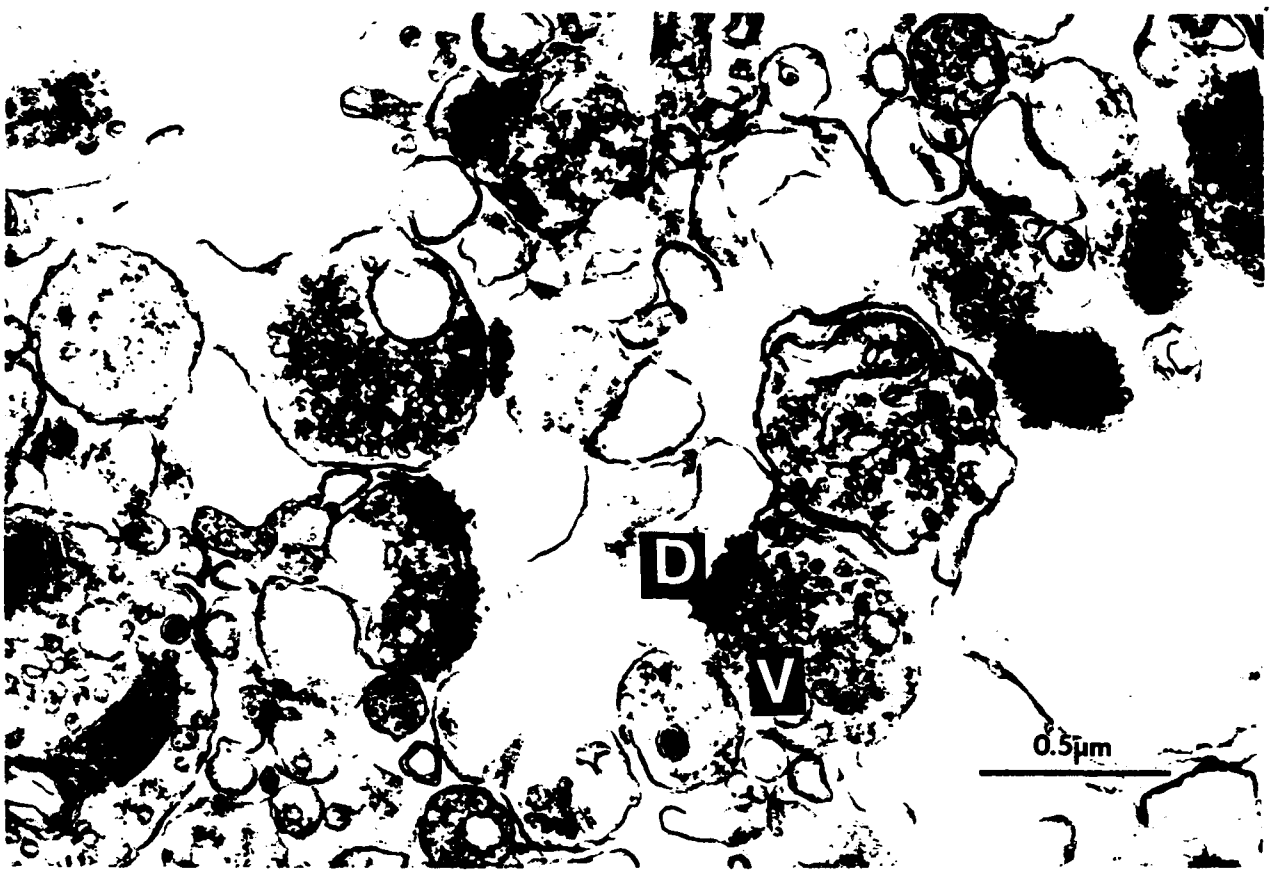

Figure 2. Transmission electron micrograph of tissue preparation, X 51,300. Numerous small vessicles (V) sited within the fragment bodies are often clustered near membrane densities (D). These features are characteristic of nerve terminal ultrastructure. The tissue preparation thus has the appearance of a debris of sheared off nerve terminals that have resealed as discrete bodies.

To begin an experiment, 1 ml of the suspension was transferred to a 5-ml glass test tube and placed in a 37°C water bath for 15 min. Then, 0.57 μCi [^{3}H]GABA (New England Nuclear, 40 Ci/mmol) diluted in 20 μl 0.01 N HCl was dispersed into the suspension, which was incubated at 37°C for an additional 5 min. Following this, the suspension was diluted by addition of 4 ml warm perfusion buffer (WPB, same as ICPB except 37°C) and drawn through a Millipore filter at 3 ml/min by a peristaltic pump. The filter assembly consisted of a Millipore Swinnex 25 polypropylene filter holder stacked from bottom to top with a 0.45 μm nylon filter (MicronSep Magna nylon 66), a microfiber glass prefilter (Millipore AP40), and glass beads (0.45-0.52 mm) to occlude dead space. The assembly was maintained at all subsequent times in a heat block thermostated to 37°C. After loading the tissue, the filter was washed with 36 ml WPB to remove unbound radioactivity. Care was taken to ensure that air was not entrained into the filter assembly at any time.

The filter assembly was placed on the superfusion apparatus and perfused at 20 drops/min (0.6 ml/min). This initial perfusion (pre-W2 perfusion*) was continued for either 5 or 25 min in two alternate experiments. During the final minute of pre-W2 perfusion, the perfusion rate was increased to 180 drops/min (5.6 ml/min). At the completion of pre-W2 perfusion, consecutive

* The symbol W2 stands for a one-minute interval of perfusion immediately preceding K^+ stimulation of the preparation. The notation is employed here to facilitate the comparison of the present data with the results reported by Gilman and colleagues (3,8). Gilman used the symbols W1 and W2 to refer to consecutive one-minute intervals preceding stimulation, and symbols 1,2,3,... to refer to one minute intervals following stimulation. Stimulation occurs at the end of interval W2 and onset of interval 1.

fractions of WPB passing across the synaptosome preparation were collected into scintillation vials for 30 s each (W2,0-30" and W2,30-60"). Perfusion was then switched from WPB to stimulation buffer (WSB, same characteristics as WPB except, in mM, NaCl 95, KCl 55, $CaCl_2$ 1.2, $MgCl_2$ 2.5). Fractions of WSB were harvested into scintillation vials for the next 5 min (15-second fractions were collected for the first 3 min following stimulation, and 30-second fractions were collected for the final 2 min).

The fractions of WPB and WSB were analyzed for ^3H activity. Scintillation cocktail (20 ml) was added to each scintillation vial for assay in a beta counter. Residual fluid in the lines of the apparatus at the end of the experiment was recovered into a scintillation vial and similarly treated. The stacked materials were recovered from the Swinnex filter holder into scintillation vials (one for glass beads, one for glass filter, and one for nylon filter), 1 ml 1% SDS solution was added to each vial as a detergent to release residual activity, and these vials were then similarly processed.

The release of [^3H]GABA during perfusion, represented by total ^3H activity (CPM) for each min, was expressed as a percentage of the total ^3H activity on the filter at the beginning of W2. This was calculated as the sum of activities of all fractions plus the residual activities at the end of the experiment. Thus, the activities appearing in fractions of the perfusate efflux or during consecutive intervals of time were normalized to the total activity bound to the tissue at the commencement of W2.

RESULTS

Experimental data is tabulated in Appendix A for the alternate apparatus (all plastic) and Appendix B for the hyperbaric superfusion apparatus (containing stainless steel). The data of one experiment for 25-min pre-W2 perfusion in the alternate apparatus, indicated by (*) in Appendix A, was considered to be possibly not valid because of technical errors. Data for another experiment for 25-min pre-W2 perfusion in the alternate apparatus, indicated by (**) in Appendix A, appeared to be grossly anomalous, although the reasons for this were not understood. These outliers were excluded from further data analysis. Twelve experiments were successfully completed for each of the treatment conditions (superfusion *vs.* alternate apparatus; 5-min *vs.* 25-min pre-W2 perfusion), except that only ten experiments were completed in the case of 25-min pre-W2 perfusion in the alternate apparatus because of the outliers.

The effects of apparatus and pre-W2 perfusion time were assessed by analysis of variance (ANOVA). A three-way ANOVA with repeated measures on one factor was utilized. The repeated measure on each experiment was taken to be the normalized activity appearing in the perfusion efflux in consecutive time intervals, reflecting the temporal profile of [^{3}H] release. To facilitate the analysis, the first two experiments of each day were excluded. This eliminated the outliers. It also simplified the statistical computations by imposing a balanced design, *i.e.*, equal numbers of experiments assigned to each treatment condition. The ANOVA was

TABLE 1. Analysis of variance for normalized release of [^{3}H] (%/100) in consecutive one-minute intervals

Source	SS	df	MS	F
Total	2.54×10^{-2}	191		
Between experiments	3.07×10^{-3}	31		
Apparatus	3.98×10^{-5}	1	3.98×10^{-5}	0.37
Pre-W2 Interval	3.21×10^{-5}	1	3.21×10^{-5}	0.30
Pre-W2 x Apparatus	2.75×10^{-5}	1	2.75×10^{-5}	0.26
$Error_b$	2.97×10^{-3}	28	1.06×10^{-4}	
Within experiments	2.23×10^{-2}	160		
Time	2.03×10^{-2}	5	4.06×10^{-3}	302.06*
Time x Apparatus	3.65×10^{-5}	5	7.30×10^{-6}	0.54
Time x Pre-W2	5.74×10^{-5}	5	1.09×10^{-5}	0.81
Time x Pre-W2 x Apparatus	2.54×10^{-5}	5	5.07×10^{-6}	0.38
$Error_w$	1.88×10^{-3}	140	1.34×10^{-5}	

* Sum and Mean Squares shown are calculated from normalized release %/100
** $p < 0.0001$

TABLE 2. Analysis of variance for normalized release of [^{3}H] in consecutive fifteen-second intervals

Source	SS*	df	MS*	F
Total	6.28×10^{-3}	383		
Between experiments	8.83×10^{-4}	31		
Apparatus	6.99×10^{-6}	1	6.99×10^{-6}	0.23
Pre-W2 Interval	1.10×10^{-5}	1	1.10×10^{-5}	0.36
Pre-W2 x Apparatus	5.75×10^{-6}	1	5.75×10^{-6}	0.19
$Error_b$	8.59×10^{-4}	28	3.07×10^{-5}	
Within experiments	5.39×10^{-3}	352		
Time	4.47×10^{-3}	11	4.07×10^{-4}	151.09**
Time x Apparatus	3.72×10^{-5}	11	3.38×10^{-6}	1.26***
Time x Pre-W2	1.87×10^{-5}	11	1.70×10^{-6}	0.63
Time x Pre-W2 x Apparatus	3.65×10^{-5}	11	3.32×10^{-6}	1.23
$Error_w$	8.29×10^{-4}	308	2.69×10^{-6}	

* Sum and Mean Squares shown are calculated from normalized release %/100
** $p < 0.0001$
*** $p < 0.25$

thus made on a total of thirty-two experiments, eight each for the combinations of two treatment levels of two experimental variables: apparatus (alternate plastic apparatus *vs.* stainless steel hyper baric apparatus) and pre-W2 perfusion (5-min *vs.* 25-min).

Table 1 summarizes the ANOVA for normalized activity in the perfusion media collected during consecutive 1-min intervals commencing 1 min before stimulation. Temporal order was observed to be statistically significant at a high confidence level ($p < 0.0001$). The effects of type of apparatus (hyperbaric superfusion apparatus containing stainless steel *vs.* alternate plastic apparatus) and pre-W2 perfusion (5- *vs.* 25-min) were both not statistically significant, nor were the interactions between these variables with each other or with temporal order significant. Figure 3 shows the temporal profile of the mean normalized release (using all data except the outliers) of [^{3}H] for consecutive 1-min intervals. For comparison, release curves redrawn from the graphs published by Gilman and colleagues (3,8) are also displayed.

Analysis of variance (balanced design following data exclusions, as previously described) was also performed for the data from observations over twelve consecutive 15-second intervals commencing from the onset of stimulation. This analysis is summarized in Table 2. Again, the temporal profile of normalized [^{3}H] release was observed to be highly significant. The temporal profile of the mean normalized release of [^{3}H] for consecutive 15-second intervals (using all data except the outliers) is displayed in Figure 4. For this release curve, the dead volume of the

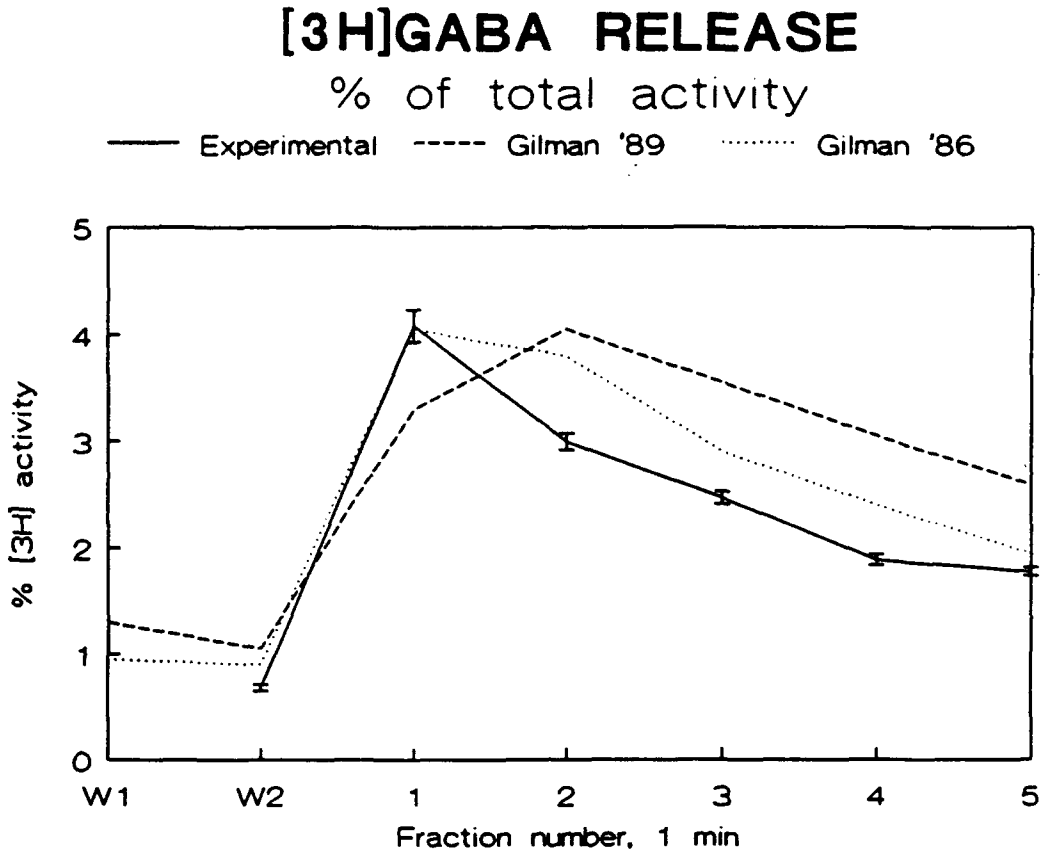

Figure 3. Normalized [^{3}H]GABA release (mean ± SEM; n = 46) during consecutive one-minute intervals (solid curve). Stimulation occurs at the end of interval W2. Normobaric [^{3}H]GABA release curves redrawn from graphs published by Gilman and colleagues (3,8) are shown for comparison (broken curves).

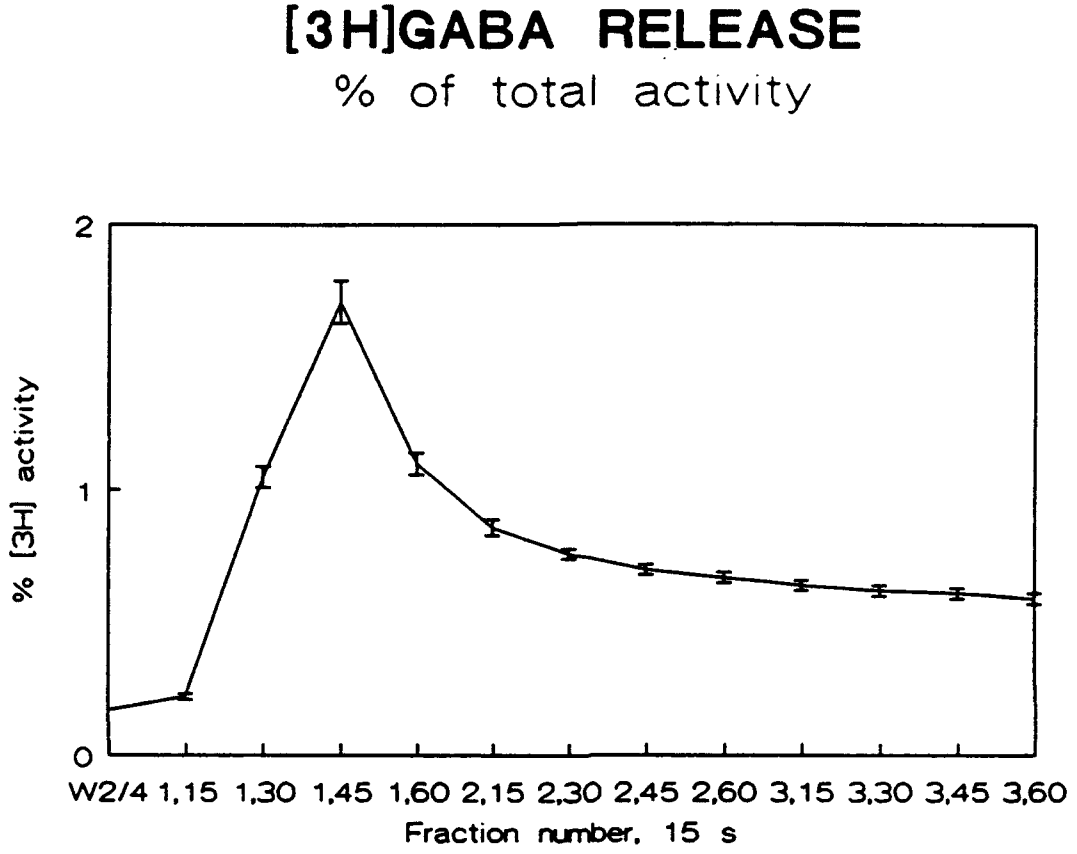

Figure 4. Normalized [^{3}H]GABA release (mean ± SEM; n = 46) during consecutive fifteen-second intervals. Basal release is shown as W2/4, one fourth of the normalized release in the one-minute interval immediately preceding stimulation. Stimulation occurs at the end of interval W2. Time intervals are shown as min,s at end mark.

perfusion apparatus beyond the tissue preparation and prior to the efflux collection point, which measured to be 1.1 ml, becomes apparent. This dead volume required approximately 12 s to elute atthe given perfusion flow rate, so it was contained in the collection of the first 15-second fraction of perfusate following stimulation.

Though not achieving a conventional ($p < 0.05$) level of statistical significance, the ANOVA for the 15-second data suggested a possible interaction between the temporal order of the collected fractions and the perfusion apparatus employed. However, further inspection of the means of normalized [^{3}H] release on consecutive 15-second intervals for each apparatus suggested a slightly *higher* trend of release while using the hyperbaric superfusion (*i.e.*, stainless steel) apparatus, particularly in the midportion of the release curve during the second minute after stimulation.

DISCUSSION

A strongly significant effect in the temporal order of collected fractions of perfusate is in accordance with expectation: Increased efflux of [^{3}H]GABA *should* occur from viable synaptosomes following stimulation. Figures 1 and 2 demonstrate ultrastructural features consistent with nerve terminal endings, and Figure 3 indicates the intact functional properties of the preparation to take up [^{3}H]GABA during incubation and to release it upon depolarization with K^+. Taken together, these results strongly indicate that the methodology to prepare the intended isolated nerve terminals was successful. Moreover, the observed release curve (Figure 3) in comparison with the previous data from Gilman and colleagues suggests that the functional characteristics of the several tissue preparations were qualitatively very similar, notwithstanding different methods, times, investigators, and so on. This inspires confidence in the capability to replicate the phenomena previously investigated and in the comparability of results, even though there were substantial differences in the procedures because of constraints and limitations of the respective equipment in the different experiments. Most noteworthy is that the perfusion flow rates utilized by Gilman and colleagues were considerably lower than for the present study. Evidently this change did not appreciably affect the release properties of the preparation while using [^{3}H]GABA.

The absence of a statistically significant difference in the release phenomena observed while using superfusion devices

constructed of different materials suggests that stainless steel did not exert any experimentally important toxic effect. Indeed the indication, albeit nonsignificant, of a possible interaction between temporal order of fractions of perfusate and apparatus reveals that the release while using the stainless steel apparatus was slightly *more* robust. Nor did a five-fold increase, from 5 min to 25 min, in the contact time of the preparation with the apparatus before collection of the basal prestimulation fractions of perfusate exert any effect on the normalized release curves. The preparation did not appear to experience any poisoning during the additional contact exposure, nor was there any "run-down" of the functional properties of the preparation with a differential passage of time of this degree.

Finally, it is to be noted that the normalization of the [^{3}H] release was calculated using a denominator which was the total activity bound to the preparation at the *end* of the 5- or 25-min perfusion preinterval, vice the *beginning*.

CONCLUSIONS

The phenomena of K^+-stimulated release of [^{3}H] activity from guinea pig cerebrocortical synaptosomes loaded with [^{3}H]GABA, as previously investigated by Gilman and colleagues (3,8), was replicated under normobaric conditions while using a hyperbaric superfusion apparatus of new design. The results were qualitatively very similar, notwithstanding considerable differences in method, *e.g.*, increased perfusion rate. There is no evidence of any toxic effect exerted by the construction materials of the new apparatus. Therefore, such departures from the observations of Gilman and colleagues that might arise while using the new apparatus could not, in any liklihood, be ascribed to the material properties of the apparatus, *per se*.

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APPENDIX A

TABULATION OF EXPERIMENTAL DATA

Experiments performed using the alternate superfusion apparatus.

For each date shown, six experiments were performed. The data for each experiment is tabulated in a column. The serial order of each experiment is indicated as the experiment number at the head of each column. Pre-W2 interval is an experimental variable, the amount of elapsed time from installation of the tissue preparation on the perfusion apparatus to beginning the collection the W2 fraction of perfusate. For each experiment, 18 consecutive fractions of perfusate were collected: 2 thirty-second fractions prior to stimulation, W2,0-30" and W2,30-60"; and 12 fifteen-second and 4 thirty-second fractions following stimulation, 1, 0-15" through 5, 30-60". Whole numbers indicate [^{3}H] activity in counts per minute (CPM) which appeared in these fractions. After collection of the final fraction, perfusion was terminated. [^{3}H] activity remaining on glass beads, glass filter, nylon filter, and in the residual fluid in the perfusion apparatus are shown respectively. TOTAL is the column sum and reflects the total [^{3}H] activity on the superfusion apparatus at the beginning of W2.

For each Fraction, the proportion of [^{3}H] activity as a percent of TOTAL is indicated as a %. Activities are thus normalized to the TOTAL activity for each experiment. Normalized values are also tabulated for equal intervals of time. For example, % for Interval W2 is the sum of %'s of Fractions W2,0-30" and W2,30-60". It is the normalized activity that appeared in the perfusate efflux during the minute immediately preceding stimulation. Interval 1, showing the sum of %'s for Fractions 1, 0-15" through 1, 45-60", is for the consecutive minute beginning at stimulation, and so on. These normalized values for equal consecutive time intervals were used for the statistical analysis of the data.

Experiment # 1 on 20 Dec 90, indicated by *, is considered to be possibly unreliable due to technical errors. Experiment # 1 on 27 Dec 90, indicated by **, is an outlier for unknown reasons. The data of these experiments was not utilized in the statistical analyses.

Data for experiments performed 20 Dec 90

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1*	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	549	517	502	540	417	360
W2,30-60"	432	486	485	505	434	381
1, 0-15"	293	312	284	303	274	251
1, 15-30"	1695	2440	3319	1573	1533	1673
1, 30-45"	4132	3692	4277	2883	4822	3404
1, 45-60"	2366	1982	2140	2774	2597	2141
2, 0-15"	1678	1603	1924	1891	1855	1515
2, 15-30"	1390	1370	1454	1393	1463	1281
2, 30-45"	1254	1226	1349	1277	1290	1055
2, 45-60"	1281	1164	1233	1217	1278	1005
3, 0-15"	1134	1214	1226	1172	1219	1055
3, 15-30"	1101	1210	1261	1038	1166	937
3, 30-45"	1110	1167	1229	1108	1059	971
3, 45-60"	1054	1149	1172	989	1088	896
4, 0-30"	1738	2005	1965	1655	1891	1470
4, 30-60"	1590	1792	1881	1574	1817	1383
5, 0-30"	1466	1726	1847	1352	1734	1543
5, 30-60"	1537	1538	1800	1360	1579	1427
Beads	33690	51680	48939	23247	39639	32110
GlFilt	14516	16258	4126	32784	18210	82108
NyFilt	54354	58181	98938	33008	57089	19875
ResFl	1123	789	1077	911	942	730
TOTAL:	129483	153501	182428	114554	143396	157571
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.42%	0.34%	0.28%	0.47%	0.29%	0.23%
W2,30-60"	0.33%	0.32%	0.27%	0.44%	0.30%	0.24%
1, 0-15"	0.23%	0.20%	0.16%	0.26%	0.19%	0.16%
1, 15-30"	1.31%	1.59%	1.82%	1.37%	1.07%	1.06%
1, 30-45"	3.19%	2.41%	2.34%	2.52%	3.36%	2.16%
1, 45-60"	1.83%	1.29%	1.17%	2.42%	1.81%	1.36%
2, 0-15"	1.30%	1.04%	1.05%	1.65%	1.29%	0.96%
2, 15-30"	1.07%	0.89%	0.80%	1.22%	1.02%	0.81%
2, 30-45"	0.97%	0.80%	0.74%	1.11%	0.90%	0.67%
2, 45-60"	0.99%	0.76%	0.68%	1.06%	0.89%	0.64%
3, 0-15"	0.88%	0.79%	0.67%	1.02%	0.85%	0.67%
3, 15-30"	0.85%	0.79%	0.69%	0.91%	0.81%	0.59%
3, 30-45"	0.86%	0.76%	0.67%	0.97%	0.74%	0.62%
3, 45-60"	0.81%	0.75%	0.64%	0.86%	0.76%	0.57%
4, 0-30"	1.34%	1.31%	1.08%	1.44%	1.32%	0.93%
4, 30-60"	1.23%	1.17%	1.03%	1.37%	1.27%	0.88%
5, 0-30"	1.13%	1.12%	1.01%	1.18%	1.21%	0.98%
5, 30-60"	1.19%	1.00%	0.99%	1.19%	1.10%	0.91%

Data for experiments performed 20 Dec 90

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1*	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.76%	0.65%	0.54%	0.91%	0.59%	0.47%
1	6.55%	5.49%	5.49%	6.58%	6.43%	4.74%
2	4.33%	3.49%	3.27%	5.04%	4.10%	3.08%
3	3.40%	3.09%	2.68%	3.76%	3.16%	2.45%
4	2.57%	2.47%	2.11%	2.82%	2.59%	1.81%
5	2.32%	2.13%	2.00%	2.37%	2.31%	1.88%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.42%	0.34%	0.28%	0.47%	0.29%	0.23%
W2,30-60"	0.33%	0.32%	0.27%	0.44%	0.30%	0.24%
1, 0-30"	1.54%	1.79%	1.98%	1.64%	1.26%	1.22%
1, 30-60"	5.02%	3.70%	3.52%	4.94%	5.17%	3.52%
2, 0-30"	2.37%	1.94%	1.85%	2.87%	2.31%	1.77%
2, 30-60"	1.96%	1.56%	1.42%	2.18%	1.79%	1.31%
3, 0-30"	1.73%	1.58%	1.36%	1.93%	1.66%	1.26%
3, 30-60"	1.67%	1.51%	1.32%	1.83%	1.50%	1.18%
4, 0-30"	1.34%	1.31%	1.08%	1.44%	1.32%	0.93%
4, 30-60"	1.23%	1.17%	1.03%	1.37%	1.27%	0.88%
5, 0-30"	1.13%	1.12%	1.01%	1.18%	1.21%	0.98%
5, 30-60"	1.19%	1.00%	0.99%	1.19%	1.10%	0.91%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.23%	0.20%	0.16%	0.26%	0.19%	0.16%
1, 15-30"	1.31%	1.59%	1.82%	1.37%	1.07%	1.06%
1, 30-45"	3.19%	2.41%	2.34%	2.52%	3.36%	2.16%
1, 45-60"	1.83%	1.29%	1.17%	2.42%	1.81%	1.36%
2, 0-15"	1.30%	1.04%	1.05%	1.65%	1.29%	0.96%
2, 15-30"	1.07%	0.89%	0.80%	1.22%	1.02%	0.81%
2, 30-45"	0.97%	0.80%	0.74%	1.11%	0.90%	0.67%
2, 45-60"	0.99%	0.76%	0.68%	1.06%	0.89%	0.64%
3, 0-15"	0.88%	0.79%	0.67%	1.02%	0.85%	0.67%
3, 15-30"	0.85%	0.79%	0.69%	0.91%	0.81%	0.59%
3, 30-45"	0.86%	0.76%	0.67%	0.97%	0.74%	0.62%
3, 45-60"	0.81%	0.75%	0.64%	0.86%	0.76%	0.57%

Data for experiments performed 27 Dec 90

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1**	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	591	580	692	529	550	529
W2,30-60"	569	545	820	517	529	595
1, 0-15"	368	410	469	314	328	372
1, 15-30"	1551	1690	1853	56381	1838	1498
1, 30-45"	2455	2255	2413	94726	2341	2167
1, 45-60"	1698	1546	1578	18951	1484	1437
2, 0-15"	1359	1185	1445	7236	1323	1211
2, 15-30"	1207	1227	1352	3909	1143	1039
2, 30-45"	1146	1167	1206	2494	1116	997
2, 45-60"	1081	1069	1185	1790	1075	1015
3, 0-15"	1008	998	1149	1316	973	995
3, 15-30"	982	1054	1074	1060	988	900
3, 30-45"	943	995	1078	915	893	914
3, 45-60"	888	929	1039	744	950	834
4, 0-30"	1588	1473	1694	1101	1473	1527
4, 30-60"	1544	1453	1687	818	1442	1440
5, 0-30"	1570	1453	1551	682	1509	1378
5, 30-60"	1534	1371	1550	677	1307	1349
Beads	40784	62912	41198	6069	14367	7010
GlFilt	87453	63188	51223	9332	122537	101772
NyFilt	46784	52707	52559	7007	57920	44598
ResFl	911	1431	1052	1056	1079	912
TOTAL:	198014	201638	169867	217624	217165	174489
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.30%	0.29%	0.41%	0.24%	0.25%	0.30%
W2,30-60"	0.29%	0.27%	0.48%	0.24%	0.24%	0.34%
1, 0-15"	0.19%	0.20%	0.28%	0.14%	0.15%	0.21%
1, 15-30"	0.78%	0.84%	1.09%	25.91%	0.85%	0.86%
1, 30-45"	1.24%	1.12%	1.42%	43.53%	1.08%	1.24%
1, 45-60"	0.86%	0.77%	0.93%	8.71%	0.68%	0.82%
2, 0-15"	0.69%	0.59%	0.85%	3.33%	0.61%	0.69%
2, 15-30"	0.61%	0.61%	0.80%	1.80%	0.53%	0.60%
2, 30-45"	0.58%	0.58%	0.71%	1.15%	0.51%	0.57%
2, 45-60"	0.55%	0.53%	0.70%	0.82%	0.50%	0.58%
3, 0-15"	0.51%	0.49%	0.68%	0.60%	0.45%	0.57%
3, 15-30"	0.50%	0.52%	0.63%	0.49%	0.45%	0.52%
3, 30-45"	0.48%	0.49%	0.63%	0.42%	0.41%	0.52%
3, 45-60"	0.45%	0.46%	0.61%	0.34%	0.44%	0.48%
4, 0-30"	0.80%	0.73%	1.00%	0.51%	0.68%	0.88%
4, 30-60"	0.78%	0.72%	0.99%	0.38%	0.66%	0.83%
5, 0-30"	0.79%	0.72%	0.91%	0.31%	0.69%	0.79%
5, 30-60"	0.77%	0.68%	0.91%	0.31%	0.60%	0.77%

Data for experiments performed 27 Dec 90

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1**	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.59%	0.56%	0.89%	0.48%	0.50%	0.64%
1	3.07%	2.93%	3.72%	78.29%	2.76%	3.14%
2	2.42%	2.31%	3.05%	7.09%	2.14%	2.44%
3	1.93%	1.97%	2.55%	1.85%	1.75%	2.09%
4	1.58%	1.45%	1.99%	0.88%	1.34%	1.70%
5	1.57%	1.40%	1.83%	0.62%	1.30%	1.56%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.30%	0.29%	0.41%	0.24%	0.25%	0.30%
W2,30-60"	0.29%	0.27%	0.48%	0.24%	0.24%	0.34%
1, 0-30"	0.97%	1.04%	1.37%	26.05%	1.00%	1.07%
1, 30-60"	2.10%	1.89%	2.35%	52.24%	1.76%	2.07%
2, 0-30"	1.30%	1.20%	1.65%	5.12%	1.14%	1.29%
2, 30-60"	1.12%	1.11%	1.41%	1.97%	1.01%	1.15%
3, 0-30"	1.00%	1.02%	1.31%	1.09%	0.90%	1.09%
3, 30-60"	0.92%	0.95%	1.25%	0.76%	0.85%	1.00%
4, 0-30"	0.80%	0.73%	1.00%	0.51%	0.68%	0.88%
4, 30-60"	0.78%	0.72%	0.99%	0.38%	0.66%	0.83%
5, 0-30"	0.79%	0.72%	0.91%	0.31%	0.69%	0.79%
5, 30-60"	0.77%	0.68%	0.91%	0.31%	0.60%	0.77%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.19%	0.20%	0.28%	0.14%	0.15%	0.21%
1, 15-30"	0.78%	0.84%	1.09%	25.91%	0.85%	0.86%
1, 30-45"	1.24%	1.12%	1.42%	43.53%	1.08%	1.24%
1, 45-60"	0.86%	0.77%	0.93%	8.71%	0.68%	0.82%
2, 0-15"	0.69%	0.59%	0.85%	3.33%	0.61%	0.69%
2, 15-30"	0.61%	0.61%	0.80%	1.80%	0.53%	0.60%
2, 30-45"	0.58%	0.58%	0.71%	1.15%	0.51%	0.57%
2, 45-60"	0.55%	0.53%	0.70%	0.82%	0.50%	0.58%
3, 0-15"	0.51%	0.49%	0.68%	0.60%	0.45%	0.57%
3, 15-30"	0.50%	0.52%	0.63%	0.49%	0.45%	0.52%
3, 30-45"	0.48%	0.49%	0.63%	0.42%	0.41%	0.52%
3, 45-60"	0.45%	0.46%	0.61%	0.34%	0.44%	0.48%

Data for experiments performed 28 Dec 90

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	261	356	313	246	266	223
W2,30-60"	280	326	311	219	287	248
1, 0-15"	186	187	214	150	173	158
1, 15-30"	1145	939	1073	908	1082	1028
1, 30-45"	1487	1437	1215	1351	1423	1432
1, 45-60"	917	959	812	825	846	807
2, 0-15"	730	793	644	678	712	649
2, 15-30"	674	605	567	593	624	589
2, 30-45"	613	595	544	555	580	521
2, 45-60"	589	538	543	494	530	569
3, 0-15"	509	588	473	527	566	540
3, 15-30"	555	549	531	481	511	472
3, 30-45"	507	524	493	477	544	457
3, 45-60"	499	509	492	467	516	445
4, 0-30"	836	823	854	752	811	745
4, 30-60"	791	799	754	749	828	736
5, 0-30"	748	699	750	621	822	693
5, 30-60"	805	738	768	676	800	749
Beads	12018	18198	32027	17066	5095	16264
GlFilt	44329	51290	25037	42910	43085	39988
NyFilt	22761	32391	27626	31931	31842	30441
ResFl	578	628	601	481	578	535
TOTAL:	91818	114471	96642	103157	92521	98289
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.28%	0.31%	0.32%	0.24%	0.29%	0.23%
W2,30-60"	0.30%	0.28%	0.32%	0.21%	0.31%	0.25%
1, 0-15"	0.20%	0.16%	0.22%	0.15%	0.19%	0.16%
1, 15-30"	1.25%	0.82%	1.11%	0.88%	1.17%	1.05%
1, 30-45"	1.62%	1.26%	1.26%	1.31%	1.54%	1.46%
1, 45-60"	1.00%	0.84%	0.84%	0.80%	0.91%	0.82%
2, 0-15"	0.80%	0.69%	0.67%	0.66%	0.77%	0.66%
2, 15-30"	0.73%	0.53%	0.59%	0.57%	0.67%	0.60%
2, 30-45"	0.67%	0.52%	0.56%	0.54%	0.63%	0.53%
2, 45-60"	0.64%	0.47%	0.56%	0.48%	0.57%	0.58%
3, 0-15"	0.55%	0.51%	0.49%	0.51%	0.61%	0.55%
3, 15-30"	0.60%	0.48%	0.55%	0.47%	0.55%	0.48%
3, 30-45"	0.55%	0.46%	0.51%	0.46%	0.59%	0.46%
3, 45-60"	0.54%	0.44%	0.51%	0.45%	0.56%	0.45%
4, 0-30"	0.91%	0.72%	0.88%	0.73%	0.88%	0.76%
4, 30-60"	0.86%	0.70%	0.78%	0.73%	0.89%	0.75%
5, 0-30"	0.81%	0.61%	0.78%	0.60%	0.89%	0.71%
5, 30-60"	0.88%	0.64%	0.79%	0.66%	0.86%	0.76%

Data for experiments performed 28 Dec 90

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.59%	0.60%	0.65%	0.45%	0.60%	0.48%
1	4.07%	3.08%	3.43%	3.14%	3.81%	3.48%
2	2.84%	2.21%	2.38%	2.25%	2.64%	2.37%
3	2.25%	1.90%	2.06%	1.89%	2.31%	1.95%
4	1.77%	1.42%	1.66%	1.46%	1.77%	1.51%
5	1.69%	1.26%	1.57%	1.26%	1.75%	1.47%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.28%	0.31%	0.32%	0.24%	0.29%	0.23%
W2,30-60"	0.30%	0.28%	0.32%	0.21%	0.31%	0.25%
1, 0-30"	1.45%	0.98%	1.33%	1.03%	1.36%	1.21%
1, 30-60"	2.62%	2.09%	2.10%	2.11%	2.45%	2.28%
2, 0-30"	1.53%	1.22%	1.25%	1.23%	1.44%	1.26%
2, 30-60"	1.31%	0.99%	1.12%	1.02%	1.20%	1.11%
3, 0-30"	1.16%	0.99%	1.04%	0.98%	1.16%	1.03%
3, 30-60"	1.10%	0.90%	1.02%	0.92%	1.15%	0.92%
4, 0-30"	0.91%	0.72%	0.88%	0.73%	0.88%	0.76%
4, 30-60"	0.86%	0.70%	0.78%	0.73%	0.89%	0.75%
5, 0-30"	0.81%	0.61%	0.78%	0.60%	0.89%	0.71%
5, 30-60"	0.88%	0.64%	0.79%	0.66%	0.86%	0.76%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.20%	0.16%	0.22%	0.15%	0.19%	0.16%
1, 15-30"	1.25%	0.82%	1.11%	0.88%	1.17%	1.05%
1, 30-45"	1.62%	1.26%	1.26%	1.31%	1.54%	1.46%
1, 45-60"	1.00%	0.84%	0.84%	0.80%	0.91%	0.82%
2, 0-15"	0.80%	0.69%	0.67%	0.66%	0.77%	0.66%
2, 15-30"	0.73%	0.53%	0.59%	0.57%	0.67%	0.60%
2, 30-45"	0.67%	0.52%	0.56%	0.54%	0.63%	0.53%
2, 45-60"	0.64%	0.47%	0.56%	0.48%	0.57%	0.58%
3, 0-15"	0.55%	0.51%	0.49%	0.51%	0.61%	0.55%
3, 15-30"	0.60%	0.48%	0.55%	0.47%	0.55%	0.48%
3, 30-45"	0.55%	0.46%	0.51%	0.46%	0.59%	0.46%
3, 45-60"	0.54%	0.44%	0.51%	0.45%	0.56%	0.45%

Data for experiments performed 3 Jan 91

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	321	396	303	314	262	249
W2,30-60"	288	371	308	296	323	249
1, 0-15"	203	213	176	170	180	138
1, 15-30"	1271	1328	1234	1011	1285	830
1, 30-45"	1738	1653	1519	1746	1586	1401
1, 45-60"	1123	1074	995	1107	1103	847
2, 0-15"	852	815	752	827	832	719
2, 15-30"	796	729	729	753	739	641
2, 30-45"	745	738	652	758	654	521
2, 45-60"	692	716	600	680	702	565
3, 0-15"	680	670	603	636	628	536
3, 15-30"	638	610	556	633	635	518
3, 30-45"	650	635	526	598	562	503
3, 45-60"	589	590	547	557	554	489
4, 0-30"	958	1002	975	950	949	850
4, 30-60"	992	983	874	928	950	797
5, 0-30"	880	873	867	888	888	757
5, 30-60"	865	918	835	899	785	716
Beads	12057	16392	15084	25462	2977	8865
GlFilt	54644	38075	40646	27668	43936	41566
NyFilt	36266	43075	37516	38386	31169	35298
ResFl	394	387	463	392	428	495
TOTAL:	117642	112243	106760	105659	92127	97550
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.27%	0.35%	0.28%	0.30%	0.28%	0.26%
W2,30-60"	0.24%	0.33%	0.29%	0.28%	0.35%	0.26%
1, 0-15"	0.17%	0.19%	0.16%	0.16%	0.20%	0.14%
1, 15-30"	1.08%	1.18%	1.16%	0.96%	1.39%	0.85%
1, 30-45"	1.48%	1.47%	1.42%	1.65%	1.72%	1.44%
1, 45-60"	0.95%	0.96%	0.93%	1.05%	1.20%	0.87%
2, 0-15"	0.72%	0.73%	0.70%	0.78%	0.90%	0.74%
2, 15-30"	0.68%	0.65%	0.68%	0.71%	0.80%	0.66%
2, 30-45"	0.63%	0.66%	0.61%	0.72%	0.71%	0.53%
2, 45-60"	0.59%	0.64%	0.56%	0.64%	0.76%	0.58%
3, 0-15"	0.58%	0.60%	0.56%	0.60%	0.68%	0.55%
3, 15-30"	0.54%	0.54%	0.52%	0.60%	0.69%	0.53%
3, 30-45"	0.55%	0.57%	0.49%	0.57%	0.61%	0.52%
3, 45-60"	0.50%	0.53%	0.51%	0.53%	0.60%	0.50%
4, 0-30"	0.81%	0.89%	0.91%	0.90%	1.03%	0.87%
4, 30-60"	0.84%	0.88%	0.82%	0.88%	1.03%	0.82%
5, 0-30"	0.75%	0.78%	0.81%	0.84%	0.96%	0.78%
5, 30-60"	0.74%	0.82%	0.78%	0.85%	0.85%	0.73%

Data for experiments performed 3 Jan 91

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.52%	0.68%	0.57%	0.58%	0.63%	0.51%
1	3.68%	3.80%	3.68%	3.82%	4.51%	3.30%
2	2.62%	2.67%	2.56%	2.86%	3.18%	2.51%
3	2.17%	2.23%	2.09%	2.29%	2.58%	2.10%
4	1.66%	1.77%	1.73%	1.78%	2.06%	1.69%
5	1.48%	1.60%	1.59%	1.69%	1.82%	1.51%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.27%	0.35%	0.28%	0.30%	0.28%	0.26%
W2,30-60"	0.24%	0.33%	0.29%	0.28%	0.35%	0.26%
1, 0-30"	1.25%	1.37%	1.32%	1.12%	1.59%	0.99%
1, 30-60"	2.43%	2.43%	2.35%	2.70%	2.92%	2.30%
2, 0-30"	1.40%	1.38%	1.39%	1.50%	1.71%	1.39%
2, 30-60"	1.22%	1.30%	1.17%	1.36%	1.47%	1.11%
3, 0-30"	1.12%	1.14%	1.09%	1.20%	1.37%	1.08%
3, 30-60"	1.05%	1.09%	1.01%	1.09%	1.21%	1.02%
4, 0-30"	0.81%	0.89%	0.91%	0.90%	1.03%	0.87%
4, 30-60"	0.84%	0.88%	0.82%	0.88%	1.03%	0.82%
5, 0-30"	0.75%	0.78%	0.81%	0.84%	0.96%	0.78%
5, 30-60"	0.74%	0.82%	0.78%	0.85%	0.85%	0.73%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.17%	0.19%	0.16%	0.16%	0.20%	0.14%
1, 15-30"	1.08%	1.18%	1.16%	0.96%	1.39%	0.85%
1, 30-45"	1.48%	1.47%	1.42%	1.65%	1.72%	1.44%
1, 45-60"	0.95%	0.96%	0.93%	1.05%	1.20%	0.87%
2, 0-15"	0.72%	0.73%	0.70%	0.78%	0.90%	0.74%
2, 15-30"	0.68%	0.65%	0.68%	0.71%	0.80%	0.66%
2, 30-45"	0.63%	0.66%	0.61%	0.72%	0.71%	0.53%
2, 45-60"	0.59%	0.64%	0.56%	0.64%	0.76%	0.58%
3, 0-15"	0.58%	0.60%	0.56%	0.60%	0.68%	0.55%
3, 15-30"	0.54%	0.54%	0.52%	0.60%	0.69%	0.53%
3, 30-45"	0.55%	0.57%	0.49%	0.57%	0.61%	0.52%
3, 45-60"	0.50%	0.53%	0.51%	0.53%	0.60%	0.50%

APPENDIX B

TABULATION OF EXPERIMENTAL DATA

Experiments performed using the hyperbaric superfusion apparatus.

For each date shown, six experiments were performed. The data for each experiment is tabulated in a column. The serial order of each experiment is indicated as the experiment number at the head of each column. Pre-W2 interval is an experimental variable, the amount of elapsed time from installation of the tissue preparation on the perfusion apparatus to the collection the W2 fraction of perfusate. For each experiment, 18 consecutive fractions of perfusate were collected: 2 thirty-second fractions prior to stimulation, W2,0-30" and W2,30-60"; and 12 fifteen-second and 4 thirty-second fractions following stimulation, 1, 0-15" through 5, 30-60". Whole numbers indicate [^{3}H] activity in counts per minute (CPM) which appeared in these fractions. After collection of the final fraction, perfusion was terminated. [^{3}H] activity remaining on glass beads, glass filter, nylon filter, and in the residual fluid in the perfusion apparatus are shown respectively. TOTAL is the column sum and reflects the total [^{3}H] activity on the superfusion apparatus at the beginning of W2.

For each Fraction, the proportion of [^{3}H] activity as a percent of TOTAL is indicated as a %. Activities are thus normalized to the TOTAL activity for each experiment. Normalized values are subsequently tabulated for equal intervals of time. For example, % for Interval W2 is the sum of %'s of Fractions W2,0-30" and W2,30-60". It is therefore the normalized activity that appeared in the perfusate efflux during the first minute. Interval 1, showing the sum of %'s for Fractions 1, 0-15" through 1, 45-60", is for the next minute, and so on. These normalized values for equal consecutive time intervals were used for the statistical analysis of the data.

Data for experiments performed 10 Jan 91

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	173	112	148	87	102	59
W2,30-60"	162	138	133	102	105	83
1, 0-15"	94	76	83	65	81	67
1, 15-30"	460	278	416	402	513	419
1, 30-45"	911	1007	770	567	914	476
1, 45-60"	633	589	517	365	566	325
2, 0-15"	436	423	361	288	360	254
2, 15-30"	402	315	320	272	368	217
2, 30-45"	366	350	292	263	316	198
2, 45-60"	316	328	251	240	283	172
3, 0-15"	285	291	249	226	294	198
3, 15-30"	319	282	259	217	230	165
3, 30-45"	306	286	258	202	260	166
3, 45-60"	309	280	236	231	254	152
4, 0-30"	444	442	402	350	412	261
4, 30-60"	462	411	375	354	398	221
5, 0-30"	468	381	359	356	414	257
5, 30-60"	429	389	373	322	406	265
Beads	12757	2391	8483	9047	3869	1972
GlFilt	9633	16867	10726	6359	13280	8261
NyFilt	12280	14722	15122	13984	11764	10900
ResFl	288	204	178	149	318	144
TOTAL:	41933	40562	40311	34448	35507	25232
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.41%	0.28%	0.37%	0.25%	0.29%	0.23%
W2,30-60"	0.39%	0.34%	0.33%	0.30%	0.30%	0.33%
1, 0-15"	0.22%	0.19%	0.21%	0.19%	0.23%	0.27%
1, 15-30"	1.10%	0.69%	1.03%	1.17%	1.44%	1.66%
1, 30-45"	2.17%	2.48%	1.91%	1.65%	2.57%	1.89%
1, 45-60"	1.51%	1.45%	1.28%	1.06%	1.59%	1.29%
2, 0-15"	1.04%	1.04%	0.90%	0.84%	1.01%	1.01%
2, 15-30"	0.96%	0.78%	0.79%	0.79%	1.04%	0.86%
2, 30-45"	0.87%	0.86%	0.72%	0.76%	0.89%	0.78%
2, 45-60"	0.75%	0.81%	0.62%	0.70%	0.80%	0.68%
3, 0-15"	0.68%	0.72%	0.62%	0.66%	0.83%	0.78%
3, 15-30"	0.76%	0.70%	0.64%	0.63%	0.65%	0.65%
3, 30-45"	0.73%	0.71%	0.64%	0.59%	0.73%	0.66%
3, 45-60"	0.74%	0.69%	0.59%	0.67%	0.72%	0.60%
4, 0-30"	1.06%	1.09%	1.00%	1.02%	1.16%	1.03%
4, 30-60"	1.10%	1.01%	0.93%	1.03%	1.12%	0.88%
5, 0-30"	1.12%	0.94%	0.89%	1.03%	1.17%	1.02%
5, 30-60"	1.02%	0.96%	0.93%	0.93%	1.14%	1.05%

Data for experiments performed 10 Jan 91

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.80%	0.62%	0.70%	0.55%	0.58%	0.56%
1	5.00%	4.81%	4.43%	4.06%	5.84%	5.10%
2	3.62%	3.49%	3.04%	3.09%	3.74%	3.33%
3	2.91%	2.81%	2.49%	2.54%	2.92%	2.70%
4	2.16%	2.10%	1.93%	2.04%	2.28%	1.91%
5	2.14%	1.90%	1.82%	1.97%	2.31%	2.07%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.41%	0.28%	0.37%	0.25%	0.29%	0.23%
W2,30-60"	0.39%	0.34%	0.33%	0.30%	0.30%	0.33%
1, 0-30"	1.32%	0.87%	1.24%	1.36%	1.67%	1.93%
1, 30-60"	3.68%	3.93%	3.19%	2.71%	4.17%	3.17%
2, 0-30"	2.00%	1.82%	1.69%	1.63%	2.05%	1.87%
2, 30-60"	1.63%	1.67%	1.35%	1.46%	1.69%	1.47%
3, 0-30"	1.44%	1.41%	1.26%	1.29%	1.48%	1.44%
3, 30-60"	1.47%	1.40%	1.23%	1.26%	1.45%	1.26%
4, 0-30"	1.06%	1.09%	1.00%	1.02%	1.16%	1.03%
4, 30-60"	1.10%	1.01%	0.93%	1.03%	1.12%	0.88%
5, 0-30"	1.12%	0.94%	0.89%	1.03%	1.17%	1.02%
5, 30-60"	1.02%	0.96%	0.93%	0.93%	1.14%	1.05%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.22%	0.19%	0.21%	0.19%	0.23%	0.27%
1, 15-30"	1.10%	0.69%	1.03%	1.17%	1.44%	1.66%
1, 30-45"	2.17%	2.48%	1.91%	1.65%	2.57%	1.89%
1, 45-60"	1.51%	1.45%	1.28%	1.06%	1.59%	1.29%
2, 0-15"	1.04%	1.04%	0.90%	0.84%	1.01%	1.01%
2, 15-30"	0.96%	0.78%	0.79%	0.79%	1.04%	0.86%
2, 30-45"	0.87%	0.86%	0.72%	0.76%	0.89%	0.78%
2, 45-60"	0.75%	0.81%	0.62%	0.70%	0.80%	0.68%
3, 0-15"	0.68%	0.72%	0.62%	0.66%	0.83%	0.78%
3, 15-30"	0.76%	0.70%	0.64%	0.63%	0.65%	0.65%
3, 30-45"	0.73%	0.71%	0.64%	0.59%	0.73%	0.66%
3, 45-60"	0.74%	0.69%	0.59%	0.67%	0.72%	0.60%

Data for experiments performed 14 Jan 91

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	1191	534	759	559	689	1082
W2,30-60"	1112	523	767	612	758	1159
1, 0-15"	691	356	448	367	455	686
1, 15-30"	1806	1273	1511	1453	1912	856
1, 30-45"	3023	2050	2200	2331	2889	1835
1, 45-60"	2328	1388	1625	1640	1963	1668
2, 0-15"	1973	1151	1437	1424	1400	1508
2, 15-30"	1778	1037	1343	1283	1171	1285
2, 30-45"	1662	968	1252	1187	1081	1161
2, 45-60"	1572	937	1200	1148	1045	1020
3, 0-15"	1412	930	1091	1085	1062	1018
3, 15-30"	1376	908	1158	1047	938	993
3, 30-45"	1323	861	1107	1025	972	986
3, 45-60"	1224	878	1039	1001	943	959
4, 0-30"	2000	1328	1625	1579	1397	1504
4, 30-60"	1885	1263	1689	1535	1439	1500
5, 0-30"	1736	1255	1468	1442	1385	1375
5, 30-60"	1640	1226	1488	1441	1321	1360
Beads	44535	35138	53289	6288	25951	98445
GlFilt	41448	58272	38835	72864	39904	11655
NyFilt	47752	50845	52325	45995	45443	32377
ResFl	1062	809	1176	888	798	1012
TOTAL:	164529	163930	168832	148194	134916	165444
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.72%	0.33%	0.45%	0.38%	0.51%	0.65%
W2,30-60"	0.68%	0.32%	0.45%	0.41%	0.56%	0.70%
1, 0-15"	0.42%	0.22%	0.27%	0.25%	0.34%	0.41%
1, 15-30"	1.10%	0.78%	0.89%	0.98%	1.42%	0.52%
1, 30-45"	1.84%	1.25%	1.30%	1.57%	2.14%	1.11%
1, 45-60"	1.41%	0.85%	0.96%	1.11%	1.45%	1.01%
2, 0-15"	1.20%	0.70%	0.85%	0.96%	1.04%	0.91%
2, 15-30"	1.08%	0.63%	0.80%	0.87%	0.87%	0.78%
2, 30-45"	1.01%	0.59%	0.74%	0.80%	0.80%	0.70%
2, 45-60"	0.96%	0.57%	0.71%	0.77%	0.77%	0.62%
3, 0-15"	0.86%	0.57%	0.65%	0.73%	0.79%	0.62%
3, 15-30"	0.84%	0.55%	0.69%	0.71%	0.70%	0.60%
3, 30-45"	0.80%	0.53%	0.66%	0.69%	0.72%	0.60%
3, 45-60"	0.74%	0.54%	0.62%	0.68%	0.70%	0.58%
4, 0-30"	1.22%	0.81%	0.96%	1.07%	1.04%	0.91%
4, 30-60"	1.15%	0.77%	1.00%	1.04%	1.07%	0.91%
5, 0-30"	1.06%	0.77%	0.87%	0.97%	1.03%	0.83%
5, 30-60"	1.00%	0.75%	0.88%	0.97%	0.98%	0.82%

Data for experiments performed 14 Jan 91

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	1.40%	0.64%	0.90%	0.79%	1.07%	1.35%
1	4.77%	3.09%	3.43%	3.91%	5.35%	3.05%
2	4.25%	2.50%	3.10%	3.40%	3.48%	3.01%
3	3.24%	2.18%	2.60%	2.81%	2.90%	2.39%
4	2.36%	1.58%	1.96%	2.10%	2.10%	1.82%
5	2.05%	1.51%	1.75%	1.95%	2.01%	1.65%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.72%	0.33%	0.45%	0.38%	0.51%	0.65%
W2,30-60"	0.68%	0.32%	0.45%	0.41%	0.56%	0.70%
1, 0-30"	1.52%	0.99%	1.16%	1.23%	1.75%	0.93%
1, 30-60"	3.25%	2.10%	2.27%	2.68%	3.60%	2.12%
2, 0-30"	2.28%	1.33%	1.65%	1.83%	1.91%	1.69%
2, 30-60"	1.97%	1.16%	1.45%	1.58%	1.58%	1.32%
3, 0-30"	1.69%	1.12%	1.33%	1.44%	1.48%	1.22%
3, 30-60"	1.55%	1.06%	1.27%	1.37%	1.42%	1.18%
4, 0-30"	1.22%	0.81%	0.96%	1.07%	1.04%	0.91%
4, 30-60"	1.15%	0.77%	1.00%	1.04%	1.07%	0.91%
5, 0-30"	1.06%	0.77%	0.87%	0.97%	1.03%	0.83%
5, 30-60"	1.00%	0.75%	0.88%	0.97%	0.98%	0.82%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.42%	0.22%	0.27%	0.25%	0.34%	0.41%
1, 15-30"	1.10%	0.78%	0.89%	0.98%	1.42%	0.52%
1, 30-45"	1.84%	1.25%	1.30%	1.57%	2.14%	1.11%
1, 45-60"	1.41%	0.85%	0.96%	1.11%	1.45%	1.01%
2, 0-15"	1.20%	0.70%	0.85%	0.96%	1.04%	0.91%
2, 15-30"	1.08%	0.63%	0.80%	0.87%	0.87%	0.78%
2, 30-45"	1.01%	0.59%	0.74%	0.80%	0.80%	0.70%
2, 45-60"	0.96%	0.57%	0.71%	0.77%	0.77%	0.62%
3, 0-15"	0.86%	0.57%	0.65%	0.73%	0.79%	0.62%
3, 15-30"	0.84%	0.55%	0.69%	0.71%	0.70%	0.60%
3, 30-45"	0.80%	0.53%	0.66%	0.69%	0.72%	0.60%
3, 45-60"	0.74%	0.54%	0.62%	0.68%	0.70%	0.58%

Data for experiments performed 16 Jan 91

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	1091	663	669	1018	582	540
W2,30-60"	1124	667	704	964	598	569
1, 0-15"	694	440	429	628	386	358
1, 15-30"	1966	1283	1296	1829	1603	1203
1, 30-45"	3058	2196	2385	3349	2427	2208
1, 45-60"	2180	1685	1802	2079	1628	1482
2, 0-15"	1805	1380	1526	1664	1370	1262
2, 15-30"	1682	1221	1386	1491	1185	1129
2, 30-45"	1611	1181	1235	1397	1119	1040
2, 45-60"	1502	1149	1210	1328	1045	988
3, 0-15"	1445	1136	1193	1266	1044	944
3, 15-30"	1459	1071	1109	1188	987	915
3, 30-45"	1311	1049	1106	1233	990	938
3, 45-60"	1308	998	1104	1203	993	889
4, 0-30"	2156	1585	1760	1825	1529	1506
4, 30-60"	2012	1470	1650	1814	1502	1430
5, 0-30"	1943	1497	1553	1786	1470	1389
5, 30-60"	1795	1384	1538	1726	1455	1378
Beads	58550	61741	28242	79654	48067	32845
GlFilt	48752	61088	97300	42713	80777	100750
NyFilt	41767	53311	54326	45357	47729	42313
ResFl	1253	913	1016	1212	961	986
TOTAL:	180464	199108	204539	196724	199447	197062
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.60%	0.33%	0.33%	0.52%	0.29%	0.27%
W2,30-60"	0.62%	0.33%	0.34%	0.49%	0.30%	0.29%
1, 0-15"	0.38%	0.22%	0.21%	0.32%	0.19%	0.18%
1, 15-30"	1.09%	0.64%	0.63%	0.93%	0.80%	0.61%
1, 30-45"	1.69%	1.10%	1.17%	1.70%	1.22%	1.12%
1, 45-60"	1.21%	0.85%	0.88%	1.06%	0.82%	0.75%
2, 0-15"	1.00%	0.69%	0.75%	0.85%	0.69%	0.64%
2, 15-30"	0.93%	0.61%	0.68%	0.76%	0.59%	0.57%
2, 30-45"	0.89%	0.59%	0.60%	0.71%	0.56%	0.53%
2, 45-60"	0.83%	0.58%	0.59%	0.68%	0.52%	0.50%
3, 0-15"	0.80%	0.57%	0.58%	0.64%	0.52%	0.48%
3, 15-30"	0.81%	0.54%	0.54%	0.60%	0.49%	0.46%
3, 30-45"	0.73%	0.53%	0.54%	0.63%	0.50%	0.48%
3, 45-60"	0.72%	0.50%	0.54%	0.61%	0.50%	0.45%
4, 0-30"	1.19%	0.80%	0.86%	0.93%	0.77%	0.76%
4, 30-60"	1.11%	0.74%	0.81%	0.92%	0.75%	0.73%
5, 0-30"	1.08%	0.75%	0.76%	0.91%	0.74%	0.70%
5, 30-60"	0.99%	0.70%	0.75%	0.88%	0.73%	0.70%

Data for experiments performed 16 Jan 91

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	1.23%	0.67%	0.67%	1.01%	0.59%	0.56%
1	4.38%	2.81%	2.89%	4.01%	3.03%	2.66%
2	3.66%	2.48%	2.62%	2.99%	2.37%	2.24%
3	3.06%	2.14%	2.21%	2.49%	2.01%	1.87%
4	2.31%	1.53%	1.67%	1.85%	1.52%	1.49%
5	2.07%	1.45%	1.51%	1.79%	1.47%	1.40%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.60%	0.33%	0.33%	0.52%	0.29%	0.27%
W2,30-60"	0.62%	0.33%	0.34%	0.49%	0.30%	0.29%
1, 0-30"	1.47%	0.87%	0.84%	1.25%	1.00%	0.79%
1, 30-60"	2.90%	1.95%	2.05%	2.76%	2.03%	1.87%
2, 0-30"	1.93%	1.31%	1.42%	1.60%	1.28%	1.21%
2, 30-60"	1.72%	1.17%	1.20%	1.39%	1.09%	1.03%
3, 0-30"	1.61%	1.11%	1.13%	1.25%	1.02%	0.94%
3, 30-60"	1.45%	1.03%	1.08%	1.24%	0.99%	0.93%
4, 0-30"	1.19%	0.80%	0.86%	0.93%	0.77%	0.76%
4, 30-60"	1.11%	0.74%	0.81%	0.92%	0.75%	0.73%
5, 0-30"	1.08%	0.75%	0.76%	0.91%	0.74%	0.70%
5, 30-60"	0.99%	0.70%	0.75%	0.88%	0.73%	0.70%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.38%	0.22%	0.21%	0.32%	0.19%	0.18%
1, 15-30"	1.09%	0.64%	0.63%	0.93%	0.80%	0.61%
1, 30-45"	1.69%	1.10%	1.17%	1.70%	1.22%	1.12%
1, 45-60"	1.21%	0.85%	0.88%	1.06%	0.82%	0.75%
2, 0-15"	1.00%	0.69%	0.75%	0.85%	0.69%	0.64%
2, 15-30"	0.93%	0.61%	0.68%	0.76%	0.59%	0.57%
2, 30-45"	0.89%	0.59%	0.60%	0.71%	0.56%	0.53%
2, 45-60"	0.83%	0.58%	0.59%	0.68%	0.52%	0.50%
3, 0-15"	0.80%	0.57%	0.58%	0.64%	0.52%	0.48%
3, 15-30"	0.81%	0.54%	0.54%	0.60%	0.49%	0.46%
3, 30-45"	0.73%	0.53%	0.54%	0.63%	0.50%	0.48%
3, 45-60"	0.72%	0.50%	0.54%	0.61%	0.50%	0.45%

Data for experiments performed 18 Jan 91

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Fraction						
[3H] activity, CPM, in perfusate						
W2,0-30"	663	491	653	674	428	496
W2,30-60"	685	524	671	726	487	520
1, 0-15"	446	334	471	504	326	328
1, 15-30"	1885	1752	2012	2696	2122	1940
1, 30-45"	3525	3053	3650	5080	3342	2778
1, 45-60"	2516	2008	2422	2893	2112	1770
2, 0-15"	1928	1664	1908	1950	1663	1394
2, 15-30"	1767	1429	1780	1760	1516	1199
2, 30-45"	1650	1241	1599	1608	1371	1169
2, 45-60"	1443	1258	1509	1630	1295	1122
3, 0-15"	1403	1204	1563	1388	1230	1078
3, 15-30"	1384	1142	1585	1346	1184	1051
3, 30-45"	1376	1142	1358	1387	1199	1030
3, 45-60"	1302	1128	1462	1346	1202	965
4, 0-30"	2020	1745	2192	2051	1818	1561
4, 30-60"	1887	1667	2100	2097	1803	1522
5, 0-30"	1902	1613	2128	2065	1764	1496
5, 30-60"	1856	1610	2080	1990	1775	1467
Beads	53526	19446	51410	41116	21204	17114
GlFilt	55479	69047	70547	50015	77146	53037
NyFilt	60582	74571	56744	55983	82108	48981
ResFl	1253	1632	979	715	1332	1079
TOTAL:	200478	189701	210823	181020	208427	143097
% of TOTAL [3H] activity appearing in each fraction						
W2,0-30"	0.33%	0.26%	0.31%	0.37%	0.21%	0.35%
W2,30-60"	0.34%	0.28%	0.32%	0.40%	0.23%	0.36%
1, 0-15"	0.22%	0.18%	0.22%	0.28%	0.16%	0.23%
1, 15-30"	0.94%	0.92%	0.95%	1.49%	1.02%	1.36%
1, 30-45"	1.76%	1.61%	1.73%	2.81%	1.60%	1.94%
1, 45-60"	1.26%	1.06%	1.15%	1.60%	1.01%	1.24%
2, 0-15"	0.96%	0.88%	0.91%	1.08%	0.80%	0.97%
2, 15-30"	0.88%	0.75%	0.84%	0.97%	0.73%	0.84%
2, 30-45"	0.82%	0.65%	0.76%	0.89%	0.66%	0.82%
2, 45-60"	0.72%	0.66%	0.72%	0.90%	0.62%	0.78%
3, 0-15"	0.70%	0.63%	0.74%	0.77%	0.59%	0.75%
3, 15-30"	0.69%	0.60%	0.75%	0.74%	0.57%	0.73%
3, 30-45"	0.69%	0.60%	0.64%	0.77%	0.58%	0.72%
3, 45-60"	0.65%	0.59%	0.69%	0.74%	0.58%	0.67%
4, 0-30"	1.01%	0.92%	1.04%	1.13%	0.87%	1.09%
4, 30-60"	0.94%	0.88%	1.00%	1.16%	0.87%	1.06%
5, 0-30"	0.95%	0.85%	1.01%	1.14%	0.85%	1.05%
5, 30-60"	0.93%	0.85%	0.99%	1.10%	0.85%	1.03%

Data for experiments performed 18 Jan 91

% of TOTAL [3H] activity appearing in equal consecutive intervals

Pre-W2 Interval:	5 MIN			25 MIN		
Experiment #:	2	4	6	1	3	5
Interval						

% of TOTAL [3H] activity in consecutive one-minute intervals

Interval	% of TOTAL					
W2	0.67%	0.54%	0.63%	0.77%	0.44%	0.71%
1	4.18%	3.77%	4.06%	6.17%	3.79%	4.76%
2	3.39%	2.95%	3.22%	3.84%	2.80%	3.41%
3	2.73%	2.43%	2.83%	3.02%	2.31%	2.88%
4	1.95%	1.80%	2.04%	2.29%	1.74%	2.15%
5	1.87%	1.70%	2.00%	2.24%	1.70%	2.07%

% of TOTAL [3H] activity in consecutive thirty-second intervals

W2,0-30"	0.33%	0.26%	0.31%	0.37%	0.21%	0.35%
W2,30-60"	0.34%	0.28%	0.32%	0.40%	0.23%	0.36%
1, 0-30"	1.16%	1.10%	1.18%	1.77%	1.17%	1.58%
1, 30-60"	3.01%	2.67%	2.88%	4.40%	2.62%	3.18%
2, 0-30"	1.84%	1.63%	1.75%	2.05%	1.53%	1.81%
2, 30-60"	1.54%	1.32%	1.47%	1.79%	1.28%	1.60%
3, 0-30"	1.39%	1.24%	1.49%	1.51%	1.16%	1.49%
3, 30-60"	1.34%	1.20%	1.34%	1.51%	1.15%	1.39%
4, 0-30"	1.01%	0.92%	1.04%	1.13%	0.87%	1.09%
4, 30-60"	0.94%	0.88%	1.00%	1.16%	0.87%	1.06%
5, 0-30"	0.95%	0.85%	1.01%	1.14%	0.85%	1.05%
5, 30-60"	0.93%	0.85%	0.99%	1.10%	0.85%	1.03%

% of TOTAL [3H] activity in consecutive fifteen-second intervals

1, 0-15"	0.22%	0.18%	0.22%	0.28%	0.16%	0.23%
1, 15-30"	0.94%	0.92%	0.95%	1.49%	1.02%	1.36%
1, 30-45"	1.76%	1.61%	1.73%	2.81%	1.60%	1.94%
1, 45-60"	1.26%	1.06%	1.15%	1.60%	1.01%	1.24%
2, 0-15"	0.96%	0.88%	0.91%	1.08%	0.80%	0.97%
2, 15-30"	0.88%	0.75%	0.84%	0.97%	0.73%	0.84%
2, 30-45"	0.82%	0.65%	0.76%	0.89%	0.66%	0.82%
2, 45-60"	0.72%	0.66%	0.72%	0.90%	0.62%	0.78%
3, 0-15"	0.70%	0.63%	0.74%	0.77%	0.59%	0.75%
3, 15-30"	0.69%	0.60%	0.75%	0.74%	0.57%	0.73%
3, 30-45"	0.69%	0.60%	0.64%	0.77%	0.58%	0.72%
3, 45-60"	0.65%	0.59%	0.69%	0.74%	0.58%	0.67%